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Application of some antioxidants for controlling neck rot disease of onion caused by *Botrytis allii* Munn

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ABSTRACT

In this study, the antifungal activity of some antioxidants against *Botrytis allii* causing neck rot disease of onion and their efficacy on the disease incidence under greenhouse and field conditions and bulb weight during storage were investigated. *In vitro*, all evaluated antioxidants significantly reduced the mycelial linear growth and biomass at all tested concentrations. Salicylic acid at 20 mM caused the highest inhibitory effect, where it caused the highest reduction of the mycelial linear growth, and biomass reached 1.07 cm and 0.046 mg. In contrast, humic acid at 20 mM caused the lowest inhibitory effect. In the greenhouse and field trials, the antioxidants significantly varied in their effectiveness on the incidence of onion neck rot disease. The efficiency increased by increasing the concentration of each tested antioxidant. Salicylic acid at 20 mM was the most effective one and caused a high reduction in disease index (DI) and disease severity, followed by pyrogallol acid, while humic acid was the lowest effective one. During storage, the antioxidants at all concentrations tested significantly varied in their effectiveness on the incidence of neck rot disease and reduction in bulb weight of onion. The efficiency also increased by increasing the concentration of each tested antioxidant. Salicylic acid at 20 Mm caused the highest efficiency, where it caused the lowest DI and bulb weight reduction to reach 28.11 and 14.75%, respectively, compared with 77.81 and 53.63% of the control. In conclusion, this study suggests that applying antioxidants such as salicylic and pyrogallol acids could be effectively used to control neck rot disease of onion as alternaive approach instead of fungicieds.

Keywords: Onion, neck rot, *Botrytis allii*, antioxidants, salicylic acid, control.

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INTRODUCTION

Onion (*Allium cepa* L.) is considered one of the most important economic vegetable crops worldwide. Onion cultivation increased considerably during the last ten years in Egypt, where the total acreage of onion in 2019 reached 209,400 feddan with a total yield of 3,081,047 tons and an average yield of 16.219 tons/feddan (FAOSTAT, 2021). Unfortunately, onion is subjected to various pathogens during all stages of onion production, which affects its health and yield (Fritsch and Friesen, 2002). In Egypt, onions are more infected with fungal diseases than other diseases due to climatic conditions suitable to infect the plant (Abdalla *et al.*, 2019). Among these fungal diseases, neck rot disease, caused by a complex of *Botrytis* spp., is an important fungal disease of onion worldwide, especially after harvesting, causing severe bulb losses during storage (Lacy and Lorbeer, 2008). The disease is mainly caused by one or more of the following species of the genus *Botrytis* included *B. aclada* Fresen (*syn. B. allii* Munn), *B. squamosa* J.C. Walker, and *B. cinerea* Pers. (Köhl *et al.*, 1991; Brewster, 1994; Jorjandi *et al.*, 2009). Most bulb losses have occurred in temperate regions, where *B. allii* was the principal species affecting onions as the common causal pathogen appeared of rotted onion, as reported by Nielsen *et al.* (2001) and du Toit *et al.* (2002). Traditional methods for controlling neck rot of onion using fungicide applications are harmful to humans and the environment. Therefore, it has become necessary to search for safe, eco-friendly alternative approaches for control plant diseases. In this respect, many investigators worldwide have been reported the antioxidant effectiveness in seed treatment, soil, and post-harvest fruit treatment against several fungal plant pathogens. The popularity of antioxidants in plant disease control is mainly due to their safety for plants, animals, and humans. In a previous study, sodium benzoate at 10 ppm exhibited a less significant decrease in the mycelial growth of *B. allii* (neck rot) and *Fusarium oxysporum* f.sp. *cepa* (basal rot) at 1, 5, and 10 ppm and it also significantly reduced the black mold, neck rot, and basal rot diseases of onion (Hanafi, Awaref *et al.*, 2001). In other studies, sodium benzoate, citric acid, and ascorbic acid decreased the growth of *B. allii* at 2., 5, and 20 ppm and significantly decreased neck-rot diseased plants (Saleh, Wagida, 2004). Also, the field treatments of transplanted Giza-20 onion cv. with some antioxidants, including salicylic acid, significantly decreased bulb rot diseases of storage onion (Saleh, Wagida, 2008). Later, salicylic acid exhibited the best effect in reducing the mycelial growth of *Aspergillus niger* and *B. allii* and was the most effective one, where it highly decreased the incidence of either black mold or neck rot diseases of onion in both greenhouse and field trials (El-Babley, Hala, 2012). In recent studies, the strawberry and grape exposure to salicylic acid at 2 mg ml^{-1} less than 24 hours protected the plants by preventing the growth of *B. cinerea*, causing gray mold, and enhancing the plant's growth (Yousif, Dina, 2019: García-Pastor, Maria *et al.*, 2020). The objective of the current study was to evaluate *in vitro* the antifungal activity of some antioxidants at different concentrations against *B. allii* causing neck rot disease of onion. The control efficacy of antioxidants on the neck rot incidence in the greenhouse and under field conditions and bulb weight during storage were also investigated.

MATERIALS AND METHODS

Preparation of *B. allii* inoculum

According to the pathogenicity test of our previous study (Salama *et al.*, 2021), the highest pathogenic isolate No. 9 of *B. allii* causing neck rot disease of onion was used in this study. The inoculum of *B. allii* was prepared by placing two disks (0.5 cm in diameter) taken from the 7-day-old culture into conical flasks containing 100 ml autoclaved potato dextrose (PD) broth medium. Then flasks were placed on a rotary checker at 3.000 rpm and 20±0.5 °C for 2 weeks. The fungal growth of each isolate was collected by filtering the growth on sterile filter paper (SFP), washed several times with sterile distilled water (SDW), and then blended in 100 ml SDW for 30 sec to get an even spore suspension using a sterilized blender. The spore suspension was adjusted to 10^4 spores ml^{-1} by a hemocytometer and then supplied with 50 mg of Carbenicillin antibiotic (Lin *et al.*, 1995).

Effect of some antioxidants on the mycelial growth of *B. allii in vitro*

The antioxidants of humic, salicylic, and pyrogallic acids were used to study their effect on the mycelial linear growth and biomass of *B. allii*. Each antioxidant chemical (40 mM stock solution)

tested was incorporated into the PDA medium before solidifying in the conical flasks to obtain the final concentrations of 10, 15, and 20 mM. The media were poured into 9.0 cm Petri plates, and the plates were inoculated in the center with 5-mm discs of *B. allii* obtained from the 7-day old culture. Then plates were incubated at 20 ^{o}C till the control plates were wholly covered with mycelium. Inoculated plates without chemicals served as a control, and four plates (replicates) were used for each treatment. The diameter of mycelial linear growth (cm) was measured. Otherwise, the incorporated PD broth medium with the same antioxidant concentrations in the conical flasks and inoculated with 5-mm *B. allii* discs were placed on a rotary shaker at 20 °C. After ten days of shake culture, the biomass was separated from the culture broth by filtration, dried for 24 h at 70 °C, weighed (mg), and calculated the means as mentioned before.

Effect of some antioxidants on the onion infection with *B. allii* in the greenhouse

In this study, the onion seedlings were treated with the antioxidants of humic, salicylic, and pyrogallic acids at different concentrations of 0, 10, 15, and 20 mM each by spraying 10 ml of each concentration seedling ten days before inoculation with *B. allii*. The following experiments were carried out in the open greenhouse of Shandaweel Island Agricultural Research Station, Sohag governorate, during the 2017/2018 and 2018/2019 growing seasons. Onion seedlings (45-day-old) of Giza-20 cv. were surface sterilized by dipping in 0.1% sodium hypochlorite solution for 3 min, washed three times with SDW, and then left for drying at room temperature (Sayed, Amany *et al*., 2014). Formalin-sterilized plastic pots (30 cm in diameter) were filled with formalin-sterilized loam soil (5.0 kg of each pot), planted with 5 seedlings per pot, and then irrigated every other day. Later 60 days of transplanting, the neck of onion bulbs were inoculated by spraying 10 ml of spore suspension using a hand atomizer (Kaufman and Lorbeer, 1967). An equal amount of SDW was applied to the neck bulbs of the control treatment. The experiment was performed with three pots (replicates) of each isolate tested in a completely randomized design. One month after inoculation, visual observations of neck rot symptoms were recorded. The individual bulbs were rated for the disease severity (DS) using a scale of 0-4: where 0 = no rot, 1= rot only close to the neck, 2 = upper third with rot, 3 = upper two thirds with rot, 4 = more than two-thirds with rot (Köhl *et al.*, 1991). The percentages of disease incidence (DI) and disease severity were then calculated using the following formulae:

$$DI\% = \text{No. of infected plants} / \text{Total plants} \times 100$$

$$DS\% = (\Sigma\ S_i \times N_i) / (4 \times N_t) \times 100$$

Where S_i is the severity ratings 0-4, N_i is the number of plants in each ratting, and N_t is the total number of rated plants.

Effect of some antioxidants on the onion infection with *B. allii* under field conditions

In this experiment, the onion seedlings were treated with antioxidants of humic, salicylic, and pyrogallic acids at different concentrations of 0.0, 10, 15, and 20 mM each by spraying 10 ml of each concentration per seedling ten days before inoculation with *B. allii*. The following experiments were conducted under field conditions and artificial infestation at the Shandaweel Island Agriculture Research Station, Sohag, Governorate, during the 2017/2018 and 2018/2019 growing seasons. Seeds of onion cultivar Giza 20 were planted in the nursery on the 25th of September for 60 days, receiving all recommended care conditions for producing onion seedlings. A complete randomized split-plot design has followed some experiments where the main plots assembled the number of antioxidants applied ten days before inoculation with *B. allii* as mentioned before, and the subplots were used for the concentrations of each treatment. Three plots 3 × 3.5 m each were used as replicates for each treatment, and non-treated plots served as control. Each plot had four rows with 60 cm apart space between rows, and 60 seedlings were planted in each row. All cultural practices recommended for onion production were carefully followed. The inoculum of *B. allii* was prepared, as mentioned before. Later 30 days of transplanting, the neck of onion bulbs were inoculated with *B. allii* by spraying 10 ml inoculum per plant using a hand atomizer. After one month of inoculation, the percentages of DI and DS have been recorded, as mentioned before. The means over the two growing seasons were then calculated.

Effect of antioxidants on neck rot incidence and bulb weight of onion during storage

At harvesting, treated and non-treated yield onion bulbs of all field experiments previously were collected (separate replicates) and stored without topping at room temperature. The bulbs were then examined after 2 and 4 months of storage to estimate the incidence of neck rot disease. The means of the incidence for all treatments of control agents during storage were then calculated. Also, the bulb's weight readings (kg) were recorded, and the reduction in the bulb weights over the control plants for all treatments of control agents was calculated. The means over the two growing seasons were then calculated and statically analyzed.

Statistical analysis

Data obtained were statistically analyzed by the MSTAT-C program version 2.10. Duncan's multiple range tests compared means, and the least significant difference (LSD) was used at the p = 0.05 level of probability described by Gomez and Gomez (1984).

RESULTS

1. Effect of some antioxidants on the mycelial growth of *B. allii in vitro*

The influence of the antioxidants humic acid, salicylic acid, and pyrogallol acid at different concentrations 0, 10, 15, and 20 mM on the mycelial linear growth and biomass of *B. allii* was also studied. Data in Table 1 and Figure 1 show that all antioxidants significantly reduced the mycelial linear growth and biomass at all tested concentrations. Furthermore, the inhibitory effect increased by increasing the concentration of each tested antioxidant. The highest inhibitory effect on the growth was detected for salicylic acid and pyrogallol, particularly at 20 mM, where salicylic acid caused the highest reduction of mycelial linear growth, and biomass reached 1.07 cm 0.046 mg compared with 8.85 cm, 0.301 mg in control, respectively. Pyrogallol acid at 20 mM caused 1.22 cm and 0.092 mg of mycelial linear growth and biomass. In contrast, humic acid at 20 mM caused the lowest inhibitory effect and reduced the mycelial linear growth and biomass to 1.70 cm and 0.184 mg, respectively, compared to the control.

Table 1 :Effect of some antioxidants on the mycelial growth of *B. allii*.

Antioxidants	Concentrations (mM)	Mycelial linear growth (cm)	Biomass (mg)
Humic acid	0	8.85	0.301
	10	3.02	0.172
	15	1.82	0.191
	20	1.70	0.184
Mean		**3.85**	**0.212**
Salicylic acid	0	8.85	0.301
	10	1.75	0.143
	15	1.35	0.052
	20	1.07	0.046
Mean		**3.26**	**0.136**
Pyrogallol acid	0	8.85	0.301
	10	1.80	0.116
	15	1.50	0.097
	20	1.22	0.092
Mean		**3.34**	**0.152**
General average		**3.48**	**0.167**
L.S.D. $_{0.05}$	Antioxidants (A)	0.266	0.032
	Concentrations (B)	0.209	0.025
	A × B	0.362	0.044

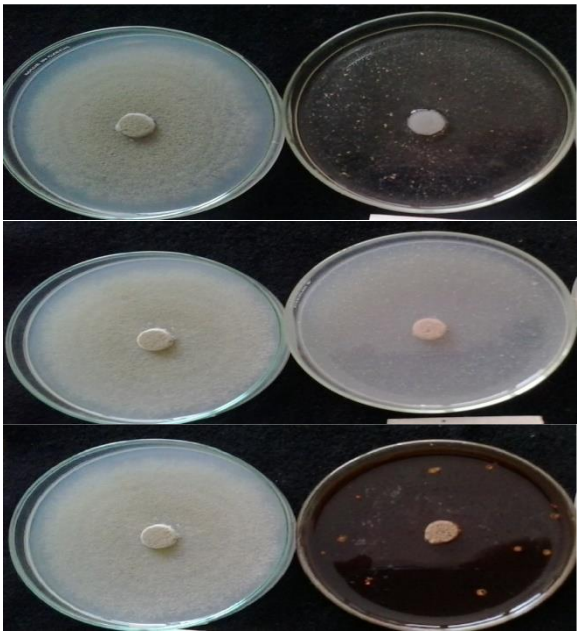

Fig. 1: Effect of some antioxidants tested at 0.3 mM on the mycelial linear growth of *B. allii in vitro*: The left plate is the control, and the right plate is Salicylic acid (A), Pyrogallol acid (B), and Humic acid (C) treatment.

2. Effect of some antioxidants on the onion infection with *B. allii* in the open greenhouse

The efficacy of three antioxidants, namely humic, salicylic, and pyrogallic acids tested at 0, 10, 15, and 20 mM on the infection with *B. allii* of onion, was studied. Data presented in Table 2 show that the antioxidants at all tested concentrations significantly varied in their effectiveness on the incidence of neck rot disease. Moreover, the efficiency increased by increasing the concentration of each tested antioxidant. Salicylic acid was the most effective one, followed by pyrogallol acid, and humic acid was the lowest effective one. The highest efficiency was detected for salicylic acid, particularly at 20 mM. It caused a high reduction in DI and DS reached 26.66 and 21.66%, respectively, compared with 95.33 and 69.66% of the control. In contrast, humic acid at 20 mM caused the lowest efficacy and slightly reduced the DI and DS to 53.33 and 43.33%, respectively. On the other hand, pyrogallic acid at 20 mM highly reduced DI and DS to 36.66 and 25.33%, respectively. However, salicylic acid was also better than pyrogallic acid in such effect.

Table 2 :Effect of some antioxidants on the onion infection with B. *allii* in the open greenhouse during the 2017/2018 and 2018/2019 growing seasons.

Antioxidants	Concentrations (mM)	DI%	DS%
Humic acid	0	95.33*	69.66
	10	80.66	53.33
	15	73.33	46.66
	20	53.33	43.33
Mean		**75.66**	**53.25**
Salicylic acid	0	95.33	69.66
	10	60.33	35.66
	15	46.66	30.33
	20	26.66	21.66
Mean		**57.25**	**39.33**
Pyrogallol acid	0	95.33	69.66
	10	66.66	39.33
	15	56.66	35.66
	20	36.66	25.33
Mean		**63.83**	**42.49**
General average		**65.58**	**45.02**
L.S.D. $_{0.05}$	Antioxidants (A)	3.93	2.98
	Concentrations (B)	3.26	2.17
	A × B	1.16	1.04

* The values of the presented data are the means over the two growing seasons.

3. Effect of some antioxidants on the onion infection with *B. allii* under filed conditions

The efficacy of three antioxidants, namely humic, salicylic, and pyrogallic acids tested at 0, 10, 15, and 20 mM on the infection with *B. allii* of onion, was studied. Data presented in Table 3 show that the antioxidants at all tested concentrations significantly varied in their effectiveness on the incidence of neck rot disease. Moreover, the efficiency increased by increasing the concentration of each tested antioxidant. In general, salicylic acid was more effective on the incidence of neck rot disease, followed by pyrogallol acid and then humic acid. The highest efficiency was detected for salicylic acid, particularly at 20 mM. It caused the highest reduction of DI and DS reached 39.66 and 23.66%, respectively, compared with 95.33 and 69.66% of the control. In contrast, humic acid at 20 mM caused the lowest efficacy and slightly reduced the DI and DS to 61.33 and 47.33%, respectively. On the other hand, pyrogallic acid at 20 mM reduced DI and DS to 49.33 and 28.66%, respectively. However, salicylic acid was also better than pyrogallic acid in such effect.

Table 3 :Effect of some antioxidants on the onion infection with *B. allii* under field conditions during the 2017/2018 and 2018/2019 growing seasons.

Antioxidants	Concentrations (mM)	DI%	DS%
Humic acid	0	93.33*	78.66
	10	82.66	63.33
	15	74.33	56.66
	20	61.33	47.33
Mean		**77.91**	**61.49**
Salicylic acid	0	93.33	78.66
	10	66.66	36.66
	15	53.33	30.33
	20	39.66	23.66
Mean		**63.25**	**42.33**
Pyrogallol acid	0	93.33	78.66
	10	73.33	55.33
	15	65.33	43.33
	20	49.33	28.66
Mean		**70.33**	**51.49**
General average		**70.49**	**51.77**
L.S.D. $_{0.05}$	Antioxidants (A)	5.76	4.88
	Concentrations (B)	3.93	3.07
	A × B	1.95	1.64

* The values of the presented data are the means over the two growing seasons.

4. Effect of antioxidants on neck rot incidence and bulb weight of onion during storage

The efficacy of three antioxidants, namely humic, salicylic, and pyrogallic acids tested at 0, 10, 15, and 20 mM on the neck rot incidence and reduction in bulb weight during storage after harvesting yield bulbs of the two growing seasons 2017/2018 and 2018/219 was studied. Data presented in Table 4 show that the antioxidants at all tested concentrations significantly varied in their effectiveness on the incidence of neck rot disease and reduction in bulb weight of onion during storage. Moreover, the efficiency increased by increasing the concentration of each tested antioxidant. The highest efficiency was detected for salicylic acid, particularly at 20 mM. It caused the lowest DI and bulb weight reduction to reach 28.11 and 14.75%, respectively, compared with 77.81 and 53.63% of the control. In contrast, humic acid at 20 mM caused the lowest efficacy and slightly reduced the DI to 46.23%, and caused a reduction of bulb weight reached 28.93%. On the other hand, pyrogallic acid at 20 mM reduced DI to 36.11% and caused a decrease of bulb weight reached 19.18%. However, salicylic acid was also better than pyrogallic acid in such effect.

Table 4 :Effect of some antioxidants on the neck rot incidence and reduction bulb weight of onion during storage after harvesting yield bulbs of the two growing seasons 2017/2018 and 2018/2019.

Antioxidants	Concentrations (mM)	DI %	Reduction in bulb weight%
Humic acid	0	77.81*	53.63
	10	57.13	37.06
	15	52.13	32.13
	20	46.23	28.93
Mean		**58.33**	**37.94**
Salicylic acid	0	77.81	53.63
	10	41.11	21.93
	15	33.66	18.18
	20	28.11	14.75
Mean		**45.17**	**27.12**
Pyrogallol acid	0	77.81	53.63
	10	49.33	29.63
	15	42.66	24.93
	20	36.11	19.18
Mean		**51.48**	**31.48**
General average		**51.66**	**32.18**
L.S.D. $_{0.05}$	Antioxidants (A)	5.76	4.08
	Concentrations (B)	3.93	3.07
	A × B	1.95	1.64

* The values of the presented data are the means over the two growing seasons.

DISCUSSION

Neck rot disease caused by *Botrytis allii* Munn is an important fungal disease of onion in Egypt and worldwide, especially after harvesting, causing severe bulb losses during storage (Köhl *et al.*, 1991; Lacy and Lorbeer, 2008; Nielsen *et al.*, 2001; Sayed, Amany *et al.*, 2014; du Toit *et al.*, 2002), in which the pathogen survives in the soil or on rotting bulbs as sclerotia (Chilvers *et al.*, 2004; Muimba-Kankolongo, 2018). In this study, the influence of the antioxidants humic acid, salicylic acid, and pyrogallol acid at different concentrations on the mycelial linear growth and biomass of *B. allii* was investigated *in vitro*. Results obtained showed that all used antioxidants significantly reduced the mycelial linear growth and biomass at all tested concentrations. Furthermore, the inhibitory effect increased by increasing the concentration of each tested antioxidant. The highest inhibitory effect on the fungus growth was detected for salicylic acid, followed by pyrogallol acid, particularly at 20 mM, where they caused the highest reduction in the mycelial linear growth and biomass. In contrast, humic acid at 20 mM caused the lowest inhibitory effect. Such results of the same or other antioxidants were also reported by Hanafi, Awaref *et al.* (2001), Shahda (2001), El-Sayed, Abeer (2004), Saleh, Wagida (2004), Hussein *et al.* (2007), Abd-El-Kareem *et al.* (2009), El-Babley, Hala (2012), Saleh, Wagida *et al.* (2013), and Yousif, Dina (2019) as showing fungicidal action. It is known that salicylic acid at higher concentrations can be very toxic to fungi because it is a natural phenolic compound contain mono hydroxybenzoic acid with an orthor and para position of OH- group (Huang *et al.*, 2009) that have an inhibitory effect on microbial pathogens and that the reason to the toxic effect on the fungus (Ansari *et al.*, 2013). Applying antioxidants to onion plants ten days before inoculation with *B. allii* significantly reduced the neck rot incidence and severity under greenhouse and field conditions. It also decreased the reduction of onion bulb weight due to infection with *B. allii* during storage compared with the untreated control plants. Salicylic acid followed by pyrogallol acid at 20 mM gave the best results, while humic acid was less effective. Such effects of the same or other antioxidants were also reported by Hanafi, Awaref *et al.* (2001), Shahda (2001), El-Sayed, Abeer

(2004), Saleh, Wagida (2004), Hussein *et al.* (2007), Abd-El-Kareem *et al.* (2009), El-Babley, Hala (2012), Saleh, Wagida *et al.* (2013), and Yousif, Dina (2019). In addition, salicylic acid has been shown to play an important role in the expression of both local plant resistance, controlled by major genes, and systemic induced resistance developed after an initial pathogen attack (Hammerschmidt and Smith-Becker, 2000). In conclusion, this study suggests that applying antioxidants such as salicylic and pyrogallol acids could be effectively used to control neck rot disease of onion as alternaive approach instead of fungicieds.

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الملخص العربى

تطبيق بعض مضادات الأكسدة لمقاومه مرض عفن الرقبه فى البصل المتسبب عن الفطر **Botrytis allii Munn**

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في هذه الدراسة، تم دراسة فعالية التضاد الفطرى لبعض مضادات الأكسدة ضد الفطر Botrytis allii المسبب لمرض عفن الرقبة في البصل ومدى فاعليتها في مقاومه المرض تحت ظروف كلا من الصوبة و الحقل وتاثيرها أيضا على وزن الأبصال أثناء التخزين. في المختبر، قللت جميع مضادات الأكسدة بشكل كبير من النمو الطولي للميسلسوم و الكتلة الحيوية للفطر عند جميع التركيزات المختبرة. تسبب حمض الساليسيليك عند 20 ملي مول في أعلى تأثير مثبط ، حيث تسبب في أقل نمو طولي للميسلسوم وكتلة الحيوية للفطر، حيث بلغت 1.07 سم و 0.046 مجم، على التوالى. في المقابل، تسبب حمض الهيوميك عند 20 ملي مول في أقل تأثير مثبط للفطر. في تجارب الصوبه والحقل، اختلفت مضادات الأكسدة المختبره بشكل كبير في فعاليتها في مقاومه مرض عفن الرقبه فى البصل. واذدادت الكفاءة لها عن طريق زيادة تركيز كل مضاد أكسدة تم اختباره. وكان حمض الساليسيليك عند 20 ملي مول هو الأكثر فعالية وتسبب في إنخفاض كبير في نسبه وشدة المرض، يليه حمض البيروجالول، بينما كان حمض الهيوميك هو الأقل فعالية. أثناء التخزين، اختلفت مضادات الأكسدة عند جميع التركيزات المختبرة بشكل كبير في فعاليتها في مقاومه مرض عفن الرقبة وايضا تاثيرها على الأنخفاض وزن الأبصال. واذدادت الفاعليه لها أيضًا عن طريق زيادة تركيز كل مضاد أكسدة تم اختباره. وأظهر حمض الساليسيليك عند 20 ملي مول أعلى فاعليه حيث قلل نسبه المرض والأنخفاض في وزن الأبصال اثناء التخذين وصل إلى 28.11 و 14.75٪ على التوالي مقارنة بـ 77.81 و 53.63٪ في النبات الغير معامله. في الختام، تقترح هذه الدراسة أن استخدام مضادات الأكسدة مثل أحماض الساليسيليك والبيروجالول يمكن أن تستخدم بشكل فعال وامن للسيطرة على مرض تعفن الرقبة في البصل كنهج بديل بدلاً من المبيدات الفطريه.